# Characterization of Fluorescence in the Marine Environment

Final Report

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Prepared by:

Charles H. Mazel
Physical Sciences Inc.
20 New England Business Center
Andover, MA  01810-1077
phone: (978) 689-0003
fax: (978) 689-3232
email: mazel@psicorp.com
http://www.psicorp.com/cobop

Prepared for:

Navy ONR
875 North Randolph Street, Suite 1425
Arlington, VA  22203-1995

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The overall goal of this project was to explore for and document the fluorescence of marine organisms, primarily on the seafloor, but also in the water column. We wish to determine the nature and distribution, both geographic and taxonomic, of the effect. The information gained has potential application to mapping and assessment of the sea floor, to studies of fundamental processes in marine biology, and to discovery of novel fluorescing proteins.

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**Summary**

The overall goal of this project was to explore for and document the fluorescence of marine organisms, primarily on the seafloor, but also in the water column. We wish to determine the nature and distribution, both geographic and taxonomic, of the effect. The information gained has potential application to mapping and assessment of the sea floor, to studies of fundamental processes in marine biology, and to discovery of novel fluorescing proteins.

This was largely a field-oriented project, supplemented by laboratory measurement and documentation. We conducted SCUBA dives at a variety of locations, using several different imaging/exploration techniques to locate instances of seafloor fluorescence. We concentrated on effects not previously observed or documented by concentrating on habitats that had not been explored much or at all, and on taxonomic groups that had not been examined in detail.

When possible we collected specimens for laboratory measurement of fluorescence excitation and emission spectra. We organized our findings in a master database that incorporates observational information (identification, location, depth, appearance, etc.), white-light and fluorescence imagery, and spectral measurements.

The discoveries made during the course of this project led to several multidisciplinary collaborations that produced important publications (*Science*). The ongoing development of the basic tools for fluorescence exploration and imaging and their commercial availability through NightSea have led to growing application of fluorescence techniques in the marine research community.

In this summary report we list: a number of the project highlights; publications and conference presentations; publications that reference fluorescence equipment in their Methods sections; and research and educational institutions using fluorescence equipment, including a number of US government laboratories.

The annual reports for this project for FY02, FY03, FY04, and FY05 are attached.

**Highlights**

Discovery and documentation of fluorescence in a wide variety of marine organisms and environments.

Measurement of fluorescence spectral properties of a wide variety of organisms.

First-ever deep sea fluorescence exploration using manned submersibles – Bahamas and Gulf of Mexico.

Discovery of fluorescence in underwater methane hydrate deposits in deep Gulf of Mexico.

Discovery of the first case of fluorescence used as a visual signal in a marine organism – paper in *Science*.

Discovery of cyanobacterial symbiosis with corals – paper in *Science*.

Development of a technique for taking fluorescence photographs in the daylight – publication in *Limnology and Oceanography: Methods*.

Prototype testing of a sequencing light source for automated differentiation of red, green, and brown algae.

Algorithm development for interpretation of multispectral fluorescing data or images.

Development of new basic lights for fluorescence exploration with transition to commercial availability.

Growing use of fluorescence exploration and imaging equipment by other researchers.

**Publications**

Mazel, C. H., 2005. Underwater fluorescence photography in the presence of ambient light. Limnol. Oceanogr. Methods, 3:499-510.

Mazel, C. H., T. W. Cronin, R. L. Caldwell, and N. J. Marshall, 2004. Fluorescent enhancement of signaling in a mantis shrimp. Science, 303:51.

Lesser, M. P., C. H. Mazel, M. Y. Gorbunov, and P. G. Falkowski, 2004. Discovery of symbiotic nitrogen-fixing cyanobacteria in corals. Science, 305:997-1000.

**Conference presentations**

Mazel, C. H., and D. G. Zawada, 2004. Diversity of fluorescence responses in benthic organisms. Oral presentation and poster presented at Ocean Optics XVII Conference, Fremantle, Australia, November 2004.

Mazel, C. H., 2002. Fluorescence imaging in the presence of ambient light – potentials, limitations, and field results. Poster presented at Ocean Optics XVI Conference, Santa Fe, NM, November 2002.

**Web site**

NightSea – http://www.nightsea.com

## Publications referencing NightSea equipment

The fluorescence exploration and imaging equipment developed under this and related contracts is contributing to, and is often the enabling factor for, a growing number of research studies. The papers listed below all reference NightSea equipment in their Methods sections.

Baird, A. H ., A. Salih, and A. Trevor-Jones, 2006. Fluorescence census techniques for the early detection of coral recruits. Coral Reefs, 25:73-76.

Gandía-Herrero, F., F. García-Carmona, and J. Escribano. 2005. Floral fluorescence effect. Nature 437:334.

Kahng, S. E., and A. Salih. 2005. Localization of fluorescent pigments in a nonbioluminescent, azooxanthellate octocoral suggests a photoprotective function. Coral Reefs, 24:435-436.

Kelmanson, I. V., and M. V. Matz, 2003. Molecular basis and evolutionary origins of color diversity in great star coral *Montastraea cavernosa* (Scleractinia: Faviida). Mol. Biol. Evol. 20:1125-1133.

Leutenegger, A., C. D'Angelo, M. V. Matz, A. Denzel, F. Oswald, A. Salih, G. U. Nienhaus, and J. Wiedenmann, 2007. It's cheap to be colorful: Anthozoans show a slow turnover of GFP-like proteins. FEBS Journal, 274: 2496–2505.

Matz, M. V., N. J. Marshall, and M. Vorobyev, 2006. Are corals colorful?. Photochemistry and Photobiology, 82:345-350.

Oswald, F., F. Schmitt, A. Leutenegger, S. Ivanchenko, C. D'Angelo, A. Salih, S. Maslakova, M. Bulina, R. Schirmbeck, G. U. Nienhaus, M. V. Matz, and J. Wiedenmann, 2007. Contributions of host and symbiont pigments to the coloration of reef corals. FEBS Journal 274: 1102-1109.

Piniak, G. A., N. D. Fogarty, C. M. Addison, and W. J. Kenworthy. 2005. Fluorescence census techniques for coral recruits. Coral Reefs, 24:496-500.

Salih, A., G. Cox, R. Szymczak, S. L. Coles, A. H. Baird, A. Dunstan, G. Cocco, J. Mills, and A. Larkum, 2006. The role of host-based color and fluorescent pigments in photoprotection and in reducing bleaching stress in corals. Proc. 10 ICRS, Okinawa.

**Research and educational institutions using NightSea equipment**

There are now at least **157** research and educational institutions in **27** countries using NightSea equipment for a wide variety of applications, and new ones are being added regularly. Besides these university, secondary education, museum, and government establishments, numerous private companies engaged in various forms of underwater and laboratory research have also purchased equipment. US government agencies are highlighted in bold.

**United States**
   * Abilene Christian University
   * American Museum of Natural History
   * Berkshire Museum
   * Bigelow Laboratory for Ocean Sciences
   * Bismarck State College
   * Boston University Marine Program
   * Brigham and Women's Hospital, Boston
   * California Institute of Technology
   * Carnegie Institution, Department of Global Ecology
   * Case Western Reserve University, Department of Orthopaedics
   * Catawba College
   * Children's Hospital, Boston
   * Columbus Zoo and Aquarium
   * Connecticut College
   * Cornell University, New York State Agricultural Experiment Station
   * Dauphin Island Sea Lab, Alabama
   * Delaware (Ohio) Area Career Center, Zoo and Aquarium School Program
   * **Environmental Protection Agency, Gulf Ecology Division**
   * **Environmental Protection Agency, Western Ecology Division**
   * The Exploratorium
   * Florida International University
   * **Flower Garden Banks National Marine Sanctuary**
   * George Mason University
   * Harbor Branch Oceanographic Institution
   * Harvard Medical School
   * Harvard School of Dental Medicine
   * Hawaii, Division of Aquatic Resources
   * Howard Hughes Medical Institute
   * Iowa State University, Department of Veterinary Microbiology
   * Joslin Diabetes Center
   * Louisiana State University, Department of Biological Sciences
   * Massachusetts General Hospital
   * Massachusetts Institute of Technology, Center for Cancer Research
   * Monterey Bay Aquarium Research Institute (MBARI)
   * Moody Gardens Aquarium (Galveston, TX)
   * Mote Marine Laboratory
   * National Center for Caribbean Coral Reef Research (NCORE)
   * **National Park Service, Biscayne National Park, Florida**
   * **National Park Service, War in the Pacific National Historic Park, Guam**

* **Naval Research Laboratory (NRL)**
* **NOAA Atlantic Oceanographic and Meteorological Laboratory**
* **NOAA Center for Coastal Environmental Health and Biomolecular Research**
* **NOAA Center for Coastal Fisheries and Habitat Research**
* **NOAA National Environmental Satellite, Data and Information Service (NOAA/NESDIS)**
* **NOAA National Marine Fisheries Service, Florida**
* **NOAA National Marine Fisheries Service, Hawaii**
* North Carolina State University, Department of Poultry Science
* Nova University
* Ocean Futures Society (Jean Michel Cousteau)
* Oceanic Institute, Hawaii
* Omaha Zoo
* Penn State University
* The Pingry School
* Princeton University, Department of Molecular Biology
* Providence College
* The Rockefeller University
* Roger Williams University
* St. Jude Children's Research Hospital
* Scripps Institution of Oceanography, Center for Marine Biodiversity and Conservation
* Scripps Institution of Oceanography, Integrative Oceanography Division
* Sea World of Orlando
* Sebastian River High School, Sebastian, Florida
* Shedd Aquarium
* Texas A&M University, Geochemical & Environmental Research Group
* Torrey Pines Institute for Molecular Studies
* Tufts University
* University of Alabama, Department of Microbiology
* University of Arizona, College of Medicine
* University of California Berkeley
* University of California Santa Barbara, Department of Chemical Engineering
* University of California Santa Barbara, Marine Science Institute
* University of California Santa Cruz
* University of Connecticut, Marine Sciences
* University of Detroit Mercy
* University of Florida, Whitney Laboratory
* University of Hawaii, Department of Oceanography
* University of Hawaii, Hawaii Institute of Marine Biology
* University of Houston, Department of Biology and Biochemistry
* University of Maine, Darling Marine Center
* University of Maryland Baltimore County
* University of Maryland, Chesapeake Biological Laboratory
* University of Maryland Medical Center
* University of Miami, Department of Microbiology and Immunology
* University of Miami, Rosenstiel School of Marine and Atmospheric Science
* University of Mississippi, Department of Pharmacognosy
* University of New Hampshire, Department of Zoology
* University of North Carolina, Chapel Hill, Institute of Marine Sciences

* University of North Carolina, Wilmington
* University of South Florida, College of Marine Science
* University of Utah
* University of Washington, Department of Comparative Medicine
* **Veterans Administration Medical Center, West Roxbury, MA**
* Wake Forest University, Department of Biology
* Washington State University, School of Biological Sciences
* Yale University, John B. Pierce Laboratory
* Yale University School of Medicine

**Australia**
* Australian Institute of Marine Science
* Garvan Institute of Medical Research
* Great Barrier Reef Marine Park Authority, ReefHQ, Townsville
* James Cook University, Centre for Coral Reef Biodiversity, Townsville
* University of Adelaide, Research Centre for Reproductive Health
* University of Queensland -- Centre for Marine Studies
* University of Queensland -- Heron Island Research Station
* University of Queensland -- Vision, Touch and Hearing Research Centre
* University of Sydney
* University of Technology, Sydney, Department of Environmental Sciences
* University of Western Australia

**Belgium**
* Catholic University of Louvain, Marine Biology Laboratory

**Bermuda**
* Bermuda Biological Station for Research

**Bonaire**
* Council on International Educational Exchange (CIEE), Study Abroad Program
* STINAPA, National Marine Park

**Canada**
* Capilano College
* Douglas Hospital, Montreal
* Parks Canada Agency, Western Canada Service Centre

**China**
* Chinese University of Hong Kong, Department of Biochemistry

**Denmark**
* Fyns Amt, Afdelingen for Natur- og Vandmiljoe
* National Environmental Research Institute
* University of Southern Denmark

**France**
  * Cousteau Society
  * Station de Biologie Marine du Muséum National d'Histoire Naturelle a Concarneau

**Germany**
  * Center for Tropical Marine Ecology, Bremen
  * Charité-Universitätsmedizin Berlin, Center for Anatomy, Institute of Cell Biology and Neurobiology
  * Max Planck Institute for Marine Microbiology
  * Universitätsklinikum Ulm, Department of Virology
  * University of Göttingen, Centre for Biodiversity and Ecology
  * University of Heidelberg, Pharmacological Institute
  * University of Kiel, Department of Physiology
  * University of Oldenburg
  * University of Ulm, Department of General Zoology and Endocrinology
  * University of Ulm, Laboratory Animals Research Unit

**Israel**
  * Interuniversity Institute of Eilat
  * Institute for Nature Conservation Research, Tel Aviv University

**Jamaica**
  * Discovery Bay Marine Laboratory

**Japan**
  * Japan Agency for Marine-Earth Science and Technology (JAMSTEC)
  * Environmental Science Research Laboratory, Central Research Institute of the Electric Power Industry (CRIEPI)
  * Kushimoto Marine Park

**Malaysia**
  * Center for Marine and Coastal Studies, Malaysian Science University

**Mexico**
  * Centro Universitario de la Costa, Campus Puerto Vallarta, Universidad de Guadalajara
  * Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México

**Netherlands**
  * Netherlands Institute for Sea Research
  * Rotterdam Zoo

**New Zealand**
  * National Institute of Water & Atmospheric Research

**Norway**
  * Trondheim Biological Station, Norwegian University of Science and Technology

**Oman**
  * Sultan Qaboos University, College of Agriculture and Marine Sciences

**Portugal**
  * Oceanário de Lisbao

**St. Vincent, West Indies**
  * Seascape Research and Education

**Singapore**
  * Tropical Marine Science Institute, National University of Singapore

**Spain**
  * Departmento Bioquímica y Biología Molecular, Universidad de Murcia

**Sweden**
  * Lund University Hospital

**Thailand**
  * Phuket Marine Biological Center

**Trinidad & Tobago**
  * University of the West Indies, Department of Life Sciences

**United Kingdom**
  * Manchester Metropolitan University
  * Napier University
  * Southampton Oceanography Center
  * University of Cambridge, CIMR Medical Genetics
  * University of Nottingham
  * University of St. Andrews

# Characterization of Fluorescence in the Marine Environment

Charles Mazel

Physical Sciences Inc., 20 New England Business Center, Andover, MA 01810

phone: (978) 689-0003     fax: (978) 689-3232     email: mazel@psicorp.com

Award Number: N0001402C0130

## LONG-TERM GOALS

This is an exploration project aimed at documenting fluorescence of seafloor marine organisms. We wish to determine the nature and distribution, both geographic and taxonomic, of the effect. The data gained has potential application to mapping and assessment of the sea floor.

## OBJECTIVES

The objectives of the FY02 effort were to conduct fieldwork at several locations; refine techniques for photographing and videotaping fluorescence; make laboratory measurements of fluorescence properties; create a database of observations, images, and measurements; and prepare for Year 2 efforts by selecting sites for additional fieldwork and planning deep-sea work.

## APPROACH

This is a field-oriented project, supplemented by laboratory measurement and documentation. We are diving at a variety of locations, using several different imaging/exploration techniques to locate instances of seafloor fluorescence. We are concentrating on effects not previously observed or documented by concentrating on habitats that have not been explored much or at all, and on taxonomic groups that have not been examined in detail. We are also examining structures such as old shipwrecks to follow up on an early report (Woodbridge, 1959) of anomalous fluorescence associated with an old wooden wreck site.

When possible we collect specimens for laboratory measurement of fluorescence excitation/emission properties. We are organizing our findings in a master database that includes comparative white-light and fluorescence imagery and spectral measurements when these are available.

We are experimenting with new techniques for imaging fluorescence. This includes the use of new underwater lights, using the lights in association with several different video camera systems to test performance, and experimenting with underwater digital fluorescence photography.

We are developing a new algorithm for interpretation of fluorescence images. This is to be applied to several different means of collecting fluorescence imagery (e.g., photography, video, laser line scan) and is intended to produce a robust approach to the interpretation that is relatively immune to variations in sensor-to-subject distance and in water optical properties.

The project is a direct collaboration with Dr. Michael Lesser, University of New Hampshire, who is funded under a separate award. Dr. Lesser is collaborating in the fieldwork and performing chemical analysis of some of the fluorescing pigments. In addition, we are forming new collaborations as the opportunity arises. These can arise when we discover a novel fluorescence in the field and contact researchers with long-term interests in those specific organisms. An example is a collaboration with several researchers with interests in mantis shrimp (see Transitions, below). Not only are our findings of interest to them, but they are also sending us new specimens, thus expanding our sampling capability beyond what we could achieve on our own.

**WORK COMPLETED**

We conducted fieldwork at locations in Hawaii, Australia, the Bahamas, and New England. The effort ranged from a few dives-of-opportunity at some locations to a concerted multi-week effort at others. Our efforts focused on 1) exploring for fluorescence and 2) experimenting with several new viewing/imaging approaches. Two of the dive sites were old shipwrecks - one a Revolutionary War era ship in Maine, and the other a canal barge in Lake Champlain, Vermont.

We compared the performance of several combinations of underwater lights and video cameras. The experimentation with underwater digital photography was very successful and demonstrated that high-quality fluorescence imaging could be carried out in the presence of moderate levels of ambient light. Because of this finding we conducted a focused modeling effort using Hydrolight to better understand the potentials and limitations of this approach.

We have formulated plans for fieldwork in the second year of the project. This will include a 2-week trip to the Pacific Northwest, where very few fluorescence dives have ever been made. We applied for and received spots in the 2003 schedule for two deep-water fluorescence exploration missions using submersibles: to the deep walls in the Bahamas using the Johnson Sea-Link submersible (Harbor Branch Oceanographic Institution), and to hydrothermal vents and nearby abyssal plains off the Pacific Northwest using Alvin (Woods Hole Oceanographic Institution).

**RESULTS**

In the course of our initial field trips we have discovered novel instances of fluorescence in several species each of: nudibranchs, polychaete worms, crustaceans (crabs and shrimp), stomatopods (mantis shrimp), molluscs, and fish. Most of these observations are supported by one or more of: still photography, videography, and measurement of spectral characteristics. Observations are added as they are acquired to a master database that records a wide variety of information, including taxonomic identification, date and location of sample, source and method of observation, and a verbal description of fluorescence characteristics. When the data are available, links are provided to white-light and fluorescence images of the subject and to spectral measurements. An example of the imagery and of the fluorescence spectral characterization for the operculum of a tulip snail (Bahamas) is shown in Figure 1.

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***Figure 1. White light (A) and fluorescence (B) photographs of the operculum of a tulip snail collected in the Bahamas. The sample appears brown under white light illumination and fluoresces with an intense greenish-yellow color over the entire surface. The spectral characterization (C) reveals the presence of two primary fluorescing substances, with excitation/emission peaks at approximately 370/460 nm and 480/540 nm.***

Through trial and error we learned that with proper control of the settings of the digital camera we were able to take high-quality fluorescence images in the presence of moderate levels of ambient light. This enabled us to take photographs that assisted in fluorescence exploration at depths as shallow as 3 m at midday, as long as the subject was in shadow. Figure 2 shows ambient-light and induced fluorescence photographs taken at 1130 at a depth of 9 m on a cloudy day in the Bahamas.
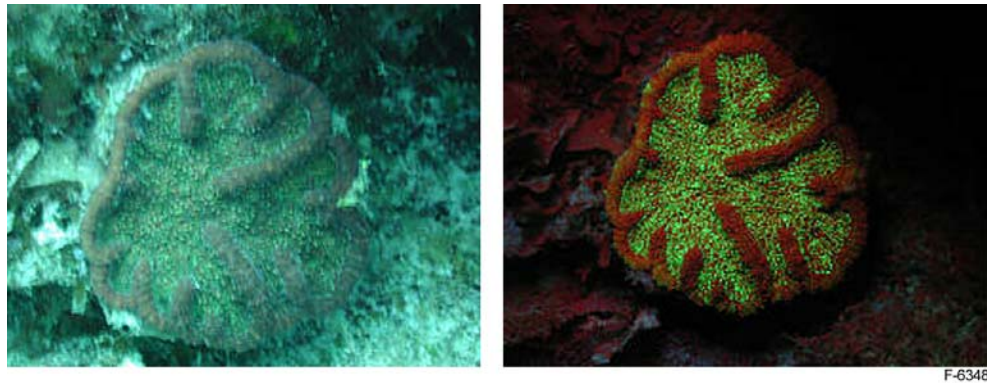


***Figure 2. Ambient light (left) and fluorescence (right) photographs of a coral on a reef wall at a depth of 9 m, Lee Stocking Island, Bahamas. Both images were made at approximately 1130. The fluorescence image clearly shows the weak red fluorescence of chlorophyll in algae and zooxanthellae, with no evidence of ambient light influence on the image.***

The Hydrolight modeling led to a better understanding of the potentials for applying this technique. Taking into consideration the combined effects of depth, time of day, cloud cover, and the fluorescence barrier filter we determined the approximate range of conditions under which daytime fluorescence photography could be carried out successfully (Figure 3). This work will be presented as a poster at the Ocean Optics XVI conference in November 2002 (Mazel (2002b).
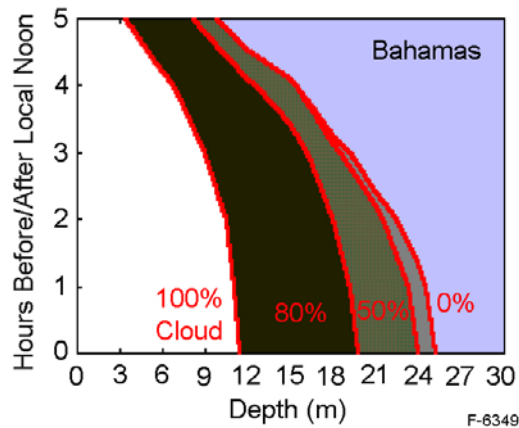
*Figure 3. Contour plots illustrating the combinations of conditions (depth and hours before/after local noon) that provide the reduction in ambient light level of six f-stops (factor of 64 reduction) needed for fluorescence photography with our digital imaging system, with cloud covers of 0, 50, 80 and 100%, for the Bahamas. For 0% cover the practical depth of approximately 24 m changes little for two hours on either side of local noon, then decreases to approximately 9 m at +/- five hours from noon. For 100% cover, the practical depth starts at 12 m at noon and shallows to almost 3 m at +/- five hours.*

## IMPACT/APPLICATIONS

The discovery of intense fluorescence in a wider array of reef organisms has implications for our interpretation of fluorescence imagery. In an effort funded as part of the Coastal Benthic Optical Properties (CoBOP) program (Mazel, 2002a) we demonstrated the potential to use multi-wavelength fluorescence imagery generated by the Fluorescence Imaging Laser Line Scanner (FILLS) to perform automated classification of coral reef surfaces (Mazel et al., 2003). In that work we interpreted all brightly fluorescing pixels as being associated with corals, even for very small targets of 1 – 5 pixels. We now know that small brightly fluorescing features can arise from polychaete worms, fish, or other sources. This will not have a large impact on estimates of percent cover, but could be very important in regard to estimation of numbers of juvenile (i.e., small) corals.

The demonstration of the ability to do fluorescence photography in the daytime will be of great value in this project, and may open the technique up for more widespread application. There are difficulties and dangers associated with night boating and diving operations that are a barrier to the use of any technique that must be carried out in darkness. An application of fluorescence that is of great current interest is in location of juvenile corals, and the ability to conduct some or all of the work in daytime would be very useful.

The novel observations of fluorescence in several taxonomic groups is generating interest among researchers who specialize in those organisms. They were unaware that fluorescence occurred and are now intrigued by the possible role that fluorescence coloration may play in the animals' behaviors. This is leading directly to new collaborations (see Transitions, below).

**TRANSITIONS**

Our discovery of fluorescence in stomatopods (mantis shrimp) made during our field effort in the Bahamas has led to a direct collaboration with leading researchers in stomatopod vision and biology (Dr. Roy Caldwell, University of California, Berkeley; Dr. Tom Cronin, University of Maryland Baltimore County; and Dr. Justin Marshall, University of Queensland, Australia). They have been sending stomatopod samples to Physical Sciences Inc. for measurement of the fluorescence of various body parts. This not only contributes to the new line of research on the stomatopods, but also to the database being assembled by this project.

In addition we are now collaborating with Dr. Neils Lindquist, University of North Carolina, on the fluorescence of isopods, and with the NOAA Center for Coastal Fisheries and Marine Habitats on application of fluorescence to research on coral recruitment.

**RELATED PROJECTS**

There is overlap between this effort and my continuing analysis of the fluorescence data collected as part of the CoBOP research program (Mazel, 2002a).

**REFERENCES**

Mazel, C. H. 2002a. CoBOP Data Analysis and Research Group Coordination. ONR FY02 Annual Report, this volume.

Mazel, C. H. 2002b. Fluorescence imaging in the presence of ambient light – potentials, limitations, and field results. Poster to be presented at Ocean Optics XVI Conference, to be held in November 2002.

Mazel, C. H., M. P. Strand, M. P. Lesser, M. P. Crosby, B. Coles, and A. J. Nevis. 2003. High resolution determination of coral reef bottom cover from multispectral fluorescence laser line scan imagery. *Limnol. Oceanogr.*, in press.

Woodbridge, R. G. III. 1959. Application of ultra-violet lights to underwater research. *Nature*: 184: 259.

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## LONG-TERM GOALS

This is an exploration project aimed at documenting fluorescence of seafloor marine organisms. We wish to determine the nature and distribution, both geographic and taxonomic, of the effect. The data gained has potential application to mapping and assessment of the sea floor.

## OBJECTIVES

The objectives of the FY03 effort were to conduct fieldwork at several locations; refine techniques for photographing and videotaping fluorescence; make laboratory measurements of fluorescence properties; create a database of observations, images, and measurements; conduct deepsea fluorescence exploration from manned submersibles; and experiment with algorithms for automated interpretation of multispectral fluorescence data.

## APPROACH

We are conducting SCUBA dives at a variety of locations, using several different imaging/exploration techniques to locate instances of seafloor fluorescence. We are concentrating on effects not previously observed or documented, by concentrating on habitats that have not been explored much or at all, and on taxonomic groups that have not been examined in detail. In this fiscal year a significant effort was devoted to outfitting a manned submersible for fluorescence search and documentation, and carrying out a series of deep exploratory dives. This part of the effort is being carried out by the Principal Investigator and by Dr. David Zawada.

When possible we collect specimens for laboratory measurement of fluorescence excitation and emission spectra. We are organizing our findings in a master database that incorporates observational information (identification, location, depth, appearance, etc.), white-light and fluorescence imagery, and spectral measurements.

We are developing approaches to computer interpretation of multispectral fluorescence data. These may be applied to several different means of collecting fluorescence data (e.g., photography, video, laser line scan) and are intended to produce robust approaches to the interpretation that are relatively immune to variations in sensor-to-subject distance and in-water optical properties. This is being done by generating sets of spectra that are based on our existing measurements of representative fluorescence emission spectra (collected during this project and from prior projects such as CoBOP) from a variety of organisms divided into 15 types. We create a large number of variations of each of the 15 spectral classes by mathematically propagating the emission through varying viewing ranges

and incorporating the effects of attenuation and spherical spreading. The resulting spectra are then divided into training and test data sets. We are experimenting with approaches including neural nets and simulated broadband detectors that use an approach along the lines of the animal visual systems comprising three or more overlapping photoreceptors. This work is being done by the Principal Investigator in collaboration with Jim Glynn and Don Frankel at PSI, both of whom have extensive experience with neural nets and algorithm development.

The project is a collaboration with Dr. Michael Lesser, University of New Hampshire, who is funded under a separate award. Dr. Lesser is collaborating in the fieldwork and is performing chemical analysis of some of the fluorescing pigments.

## WORK COMPLETED

In June we successfully conducted the first exploration for fluorescence from a submersible in deep water during a 12-day research cruise in the Bahamas aboard the R/V Seward Johnson (Harbor Branch Oceanographic Institution). We added blue and ultraviolet filters to four 400 watt high intensity lights that we placed far forward on the Johnson-SeaLink II submersible (Figure 1). A total of 18 submersible dives were made, to depths as great as 850 m (2800 ft). We made two dives a day when conditions allowed, with the first commencing at 1200 and the second at 1930. The first dive always went to at least 350 m (1200 ft) since it was necessary to reach a depth at which it was dark enough to search for fluorescence. The night dive was usually made to shallower depths, but still below the range of SCUBA operations. Fluorescing subjects were documented in situ using the submersible's video camera fitted with appropriate barrier filters, and selected specimens were collected for spectral measurement on board ship and extraction of fluorescent pigments for analysis. In addition to the submersible work we conducted a total of 54 SCUBA dives (34 day, 20 night), exploring for fluorescence and collecting specimens for spectral measurement and pigment extraction on board ship. 122 specimen observations were added to the database.



*Figure 1. View from inside the Johnson-SeaLink sphere showing the HMI lights mounted far forward on the lid of the work basket. The two inner lights are fitted with filters that transmit blue light, while the outer two are fitted with filters that transmit ultraviolet. The two pairs can be illuminated separately.To the left is the JSL video camera fitted with a yellow filter for imaging blue-light stimulated fluorescence.*

We completed the analysis of the contribution of fluorescence to the spectrum of fluorescent patches on a stomatopod (mantis shrimp), referenced in last year's report (Mazel, 2002). A manuscript on the

use of fluorescence for signaling in this animal has been accepted for publication in Science (Mazel et al., in press).

We substantially refined the format of the spectral database and are in the process of populating it with observational information, images, and spectra. There are now over 200 entries in the database.

Extensive tests were made of the approaches to creating algorithms for automated interpretation of the fluorescence data.

## RESULTS

We made the first in situ observations of fluorescence in deepwater organisms, including corals, anemones, crinoids, and others. We found that the practical requirements for searching for fluorescence were not as restrictive as originally expected. As long as the illumination spots were kept from overlapping it was possible to utilize both the blue or ultraviolet lights for fluorescence exploration and white lights for submersible operational safety at the same time. The deep-water environment at most of the dive sites was characterized by soft carbonate sediments with low density of organisms of a limited number of types, most of which did not have significant fluorescence. Brightly fluorescing animals, which were typically green-fluorescent, were easily spotted when the excitation beam passed over them.

We found that during full daylight fluorescence exploration could be conducted at depths below approximately 180 m (600 ft). At shallower depths the ambient light levels made it difficult to detect all but the brightest instances of fluorescence. The dive sites were characterized by sloping sedimented bottoms that met vertical walls at depths on the order of 300 m (1000 ft). Fluorescence was very rare on the walls until a depth of about 200 m (700 ft), at which point the orange fluorescence characteristic of encrusting phycoerythrin-containing algae began to appear (Figure 2, left). As in shallow water, the blue light filter proved more effective than the ultraviolet in stimulating fluorescence. Only a few specimens were found with the ultraviolet light that would not have been found with the blue. In addition to the deepwater observations we discovered several new instances of fluorescence in shallow-water organisms, including intense orange fluorescence in ostracods, yellow-green fluorescent amphipods, multi-color fluorescing polychaetes, and stunning magenta fluorescence in a scorpionfish (Figure 2, right).
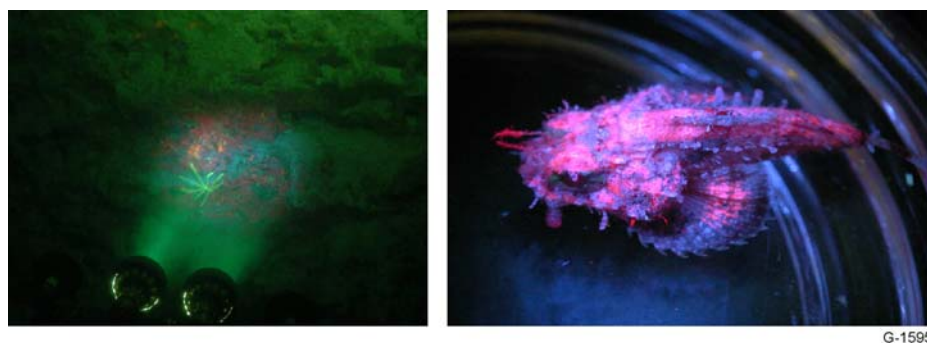


*Figure 2. Left, photograph taken from inside the Johnson-SeaLink submersible showing a pair of lights directed at a vertical wall at a depth of approximately 150 m (500 ft), revealing yellow fluorescence from a crinoid and red and orange fluorescence from encrusting algae. Right, laboratory photograph showing striking magenta fluorescence in a small (6 cm) scorpionfish collected from shallow water.*

Experimentation with new light sources for SCUBA diving led to the adoption of an off-the-shelf dive light with a high intensity discharge bulb as a primary tool for fluorescence exploration. When fitted with a custom blue interference filter (Figure 3) this makes an effective tool for searching for and videotaping fluorescence. This light-filter combination has become a popular commercial product offered through NightSea, a PSI subsidiary. We are currently experimenting with a new light that incorporates a high-intensity 1W light-emitting diode. The output of this light is comparable to that of the Light Cannon with filters, and has advantages such as smaller size, lower cost, and lower power consumption.



G-1596

*Figure 3. Underwater Kinetics Light Cannon high intensity discharge dive light fitted with a custom interference filter. The light is an effective illumination source for fluorescence exploration and close-range documentation by videotape.*

For the algorithm development, we found that dividing the spectrum into as few as six equal wavelength bands would enable near-perfect recognition of the fifteen fluorescence spectral classes we modeled (Figure 4). With fewer bands the recognition capability becomes more limited. This result has significant implications for selecting detector schemes for a general-purpose multispectral fluorescence classification system.
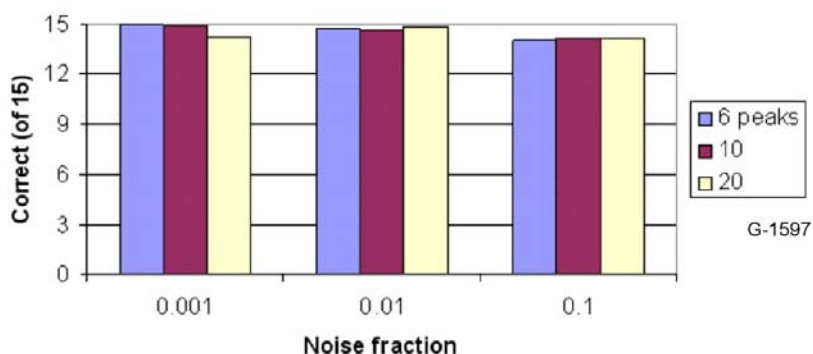


*Figure 4. Bar graph showing the performance of a neural net approach in classifying the modeled spectra in fifteen spectral classes, with three levels of added noise (0.001, 0,01, and 0.1, expressed as a fraction of maximum signal amplitude). The results show that even at the highest noise level, approaches using data divided into 6, 10 and 20 evenly spaced spectral bands produced nearly identical results, all with near-perfect classification performance.*

## IMPACT/APPLICATIONS

The continuing discovery of fluorescence phenomena in a wider array of benthic organisms has implications for our interpretation of fluorescence imagery. In an effort funded as part of the Coastal Benthic Optical Properties (CoBOP) program (Mazel, 2003) we demonstrated the potential to use multi-wavelength fluorescence imagery generated by the Fluorescence Imaging Laser Line Scanner to perform automated classification of coral reef surfaces (Mazel et al., 2003). In that work we interpreted all brightly fluorescing pixels as being associated with corals, even for very small targets of 1 to 5 pixels. We now know that small brightly fluorescing features can arise from polychaete worms, fish, or other sources. This will not have a large impact on estimates of percent cover, but could be very important in regard to estimation of numbers of juvenile (i.e., small) corals. The current results from the algorithm development show that with several additional detection bands a fluorescence system could potentially divide scenes into finer classification groups.

## TRANSITIONS

The Underwater Kinetics Light Cannon dive light fitted with the custom filters is now a standard product offered by NightSea, a subsidiary of PSI (http://www.nightsea.com/uklc.htm). The light is being used for both scientific and sport diving. On the order of 40 units have now been sold.

## RELATED PROJECTS

There is overlap between this effort and continuing analysis of the fluorescence data collected as part of the CoBOP research program (Mazel, 2003). The fluorescence data interpretation results have direct application to design of a prototype fluorescence-detecting probe being built as part of another ONR effort (Neely, 2003).

## REFERENCES

Mazel, C. H. 2002. Characterization of Fluorescence in the Marine Environment. ONR FY02 Annual Report.

Mazel, C. H. 2003. CoBOP Data Analysis and Research Group Coordination. ONR FY03 Annual Report, this volume.

Mazel, Charles H., Michael P. Strand, Michael P. Lesser, Michael P. Crosby, Bryan Coles, and Andrew J. Nevis, 2003. High resolution determination of coral reef bottom cover from multispectral fluorescence laser line scan imagery. Limnol. Oceanogr. 48:522-534.

Neely, J. 2003. Advanced Underwater Imaging. ONR FY03 Annual Report, this volume.
NightSea web site – Light Cannon page, http://www.nightsea.com/uklc.htm

## PUBLICATIONS

Mazel, C. H., T. W. Cronin, R. L. Caldwell, and N. J. Marshall.  Fluorescent Enhancement of Signaling in a Mantis Shrimp.  Science [in press, refereed].

# Characterization of Fluorescence in the Marine Environment

Charles H. Mazel

Physical Sciences Inc., 20 New England Business Center, Andover, MA 01810

phone: (978) 689-0003     fax: (978) 689-3232     email: mazel@psicorp.com

Award Number: N0001402C0130

## LONG-TERM GOALS

This is an exploration project aimed at documenting fluorescence of seafloor marine organisms. We wish to determine the nature and distribution, both geographic and taxonomic, of the effect. The data gained has potential application to mapping and assessment of the sea floor, and to discovery of novel fluorescing pigments.

## OBJECTIVES

The objectives of the FY04 effort were to continue to conduct fieldwork at several locations; refine techniques for photographing and videotaping fluorescence; make laboratory measurements of fluorescence properties; populate the database of observations, images, and measurements; conduct deepsea fluorescence exploration from manned submersibles; and experiment with algorithms for automated interpretation of multispectral fluorescence data.

## APPROACH

This is largely a field-oriented project, supplemented by laboratory measurement and documentation. We are conducting SCUBA dives at a variety of locations, using several different imaging/exploration techniques to locate instances of seafloor fluorescence. We are concentrating on effects not previously observed or documented, by concentrating on habitats that have not been explored much or at all, and on taxonomic groups that have not been examined in detail.

When possible we collect specimens for laboratory measurement of fluorescence excitation and emission spectra. We are organizing our findings in a master database that incorporates observational information (identification, location, depth, appearance, etc.), white-light and fluorescence imagery, and spectral measurements.

We are developing approaches to computer interpretation of multispectral fluorescence data. These may be applied to several different means of collecting fluorescence data (e.g., photography, video, laser line scan) and are intended to produce robust approaches to the interpretation that are relatively immune to variations in sensor-to-subject distance and in-water optical properties. We have generated sets of spectra that are based on our existing measurements of representative fluorescence emission spectra (collected during this project and from prior projects such as CoBOP) from a variety of organisms, divided into 15 types. We create a large number of variations of each of the 15 spectral classes by mathematically propagating the emission through varying viewing ranges and incorporating the effects of water column attenuation and spherical spreading. The resulting spectra are then divided

into training and test data sets. We are experimenting with several processing algorithms including neural nets (NN), a spectral angle mapper (SAM) and a Mahalanobis Distance (MD) classifier. Central to each technique is the comparison of an unknown spectrum to a reference set. In this study, the appropriate training library served as the reference. An unclassified spectrum was assigned to the class of the reference set which maximized a given similarity metric. This effort is being led by Dr. David Zawada at PSI.

The project is a collaboration with Dr. Michael Lesser, University of New Hampshire, who is funded under a separate award. Dr. Lesser is collaborating in the fieldwork and is carrying out analysis of some of the fluorescing pigments.

## WORK COMPLETED

For the fluorescence classification tests we created a training set of 1000 spectra in each of the 15 classes. These served as the reference spectra that each algorithm used to determine the class membership of each test spectrum. For a test data set we created 200 spectra in each of the 15 classes. We experimented with six different detection band sets: three were sets of bands (4, 6, and 8 bands) evenly spaced through the visible spectrum, while three were sets of bands (4, 6, and 8 bands) for which the center wavelength and spectral width were chosen based on our prior knowledge of the common spectral characteristics. All test spectra were run through each of the 6 band sets and the classification accuracy tabulated.

In addition to SCUBA diving explorations in New England, San Diego, and Bonaire, we participated in a research cruise sponsored by the NOAA Ocean Exploration program. The DeepScope (http://oceanexplorer.noaa.gov/explorations/04deepscope/welcome.html) project was a collaboration of a number of researchers exploring different aspects of light and vision in the sea. The trip afforded the opportunity make deep-water fluorescence exploration submersible dives in the Gulf of Mexico, which complements our deep Bahamas dives from last year (Mazel, 2003).

We have developed a new fluorescence excitation light source based on high intensity light-emitting diodes supplemented with our custom-made interference filters (Figure 1). This low-power (1W) light produces as much excitation light as the filtered HID light we reported on last year, with lighter weight and longer operating time.



G-8001

*Figure 1. New compact underwater flashlight based on a single 1 watt*
*high intensity light-emitting diode.*

**RESULTS**

While only a small number of submersible dives specifically devoted to fluorescence exploration could be made on the DeepScope project, they resulted in the discovery of fluorescence in a shark, a greeneye fish (*Chlorophthalmus agassizi*) (Figure 2), an anemone, a starfish, a sea urchin, and other specimens.

In addition, we found intense fluorescence in exposed methane hydrates at a hydrocarbon seep site. We were not able to take a sample for analysis, since without special sampling procedures the 'methane ice' will dissociate as it is brought to the surface.
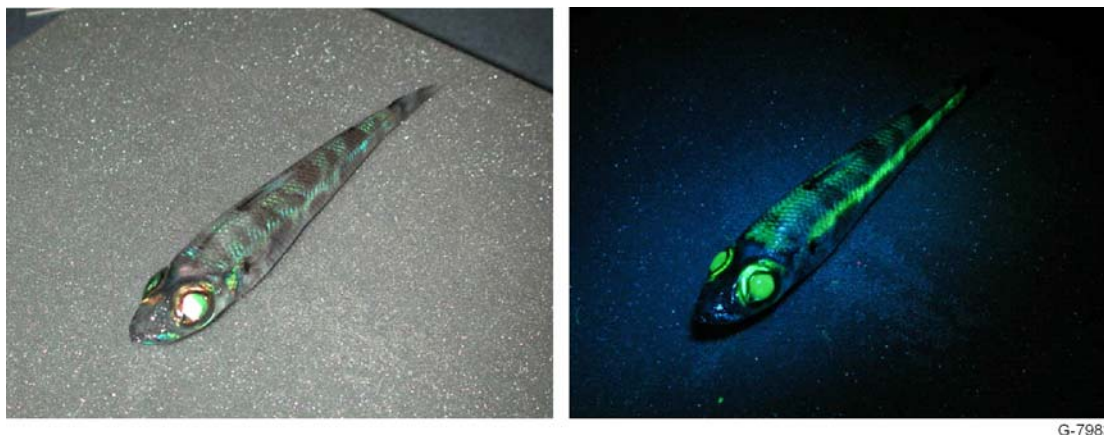


*Figure 2. White-light (left) and fluorescence photographs of the greeneye fish* **(Chlorophthalmus agassizi)** *collected at a depth of approximately 530 meters in the Gulf of Mexico. The fluorescence image reveals the intense green fluorescence of the eye, the band above the eye, and the lateral stripe.*

Figure 3 shows the classification results for the three types of algorithms. Overall, MD was the most accurate classifier; with better than 90% classification accuracy for most of the classes, while SAM was the worst. All three methods had trouble distinguishing between classes 8 and 9 (soft coral and green or brown algae). This result is not surprising since both classes have a single fluorescence peak arising from chlorophyll. The only discriminating factor is the intensity of the fluorescence. The inclusion of additional information, such as the classification of neighboring pixels, might improve the results for those classes. The most surprising result is the high degree of discrimination possible with only 4 spectral bands, especially for the MD method. Also note that the evenly-spaced band data provided almost as much discriminatory information to the MD classifier as the narrower, hand-selected bands. This finding suggests that off-the-shelf interference filters may be adequate; thus, saving the expense of fabricating custom filters.
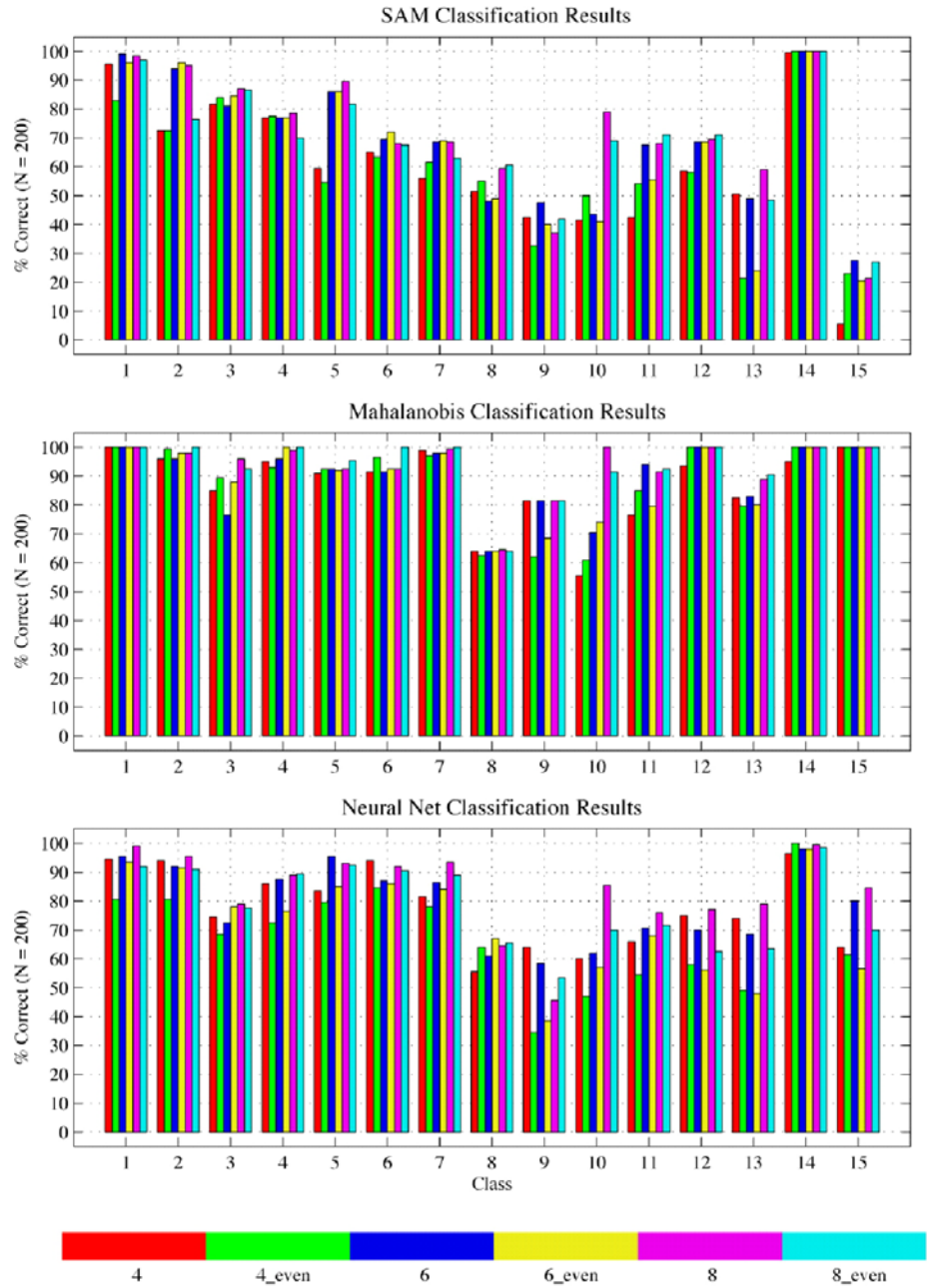
***Figure 3: Results of the algorithm classification tests. Each vertical bar represents the percentage of 200 test spectra that were correctly classified, while the color of the bar is keyed to the number and spectral placement of the simulated detection bands. The Mahalanobis Distance classifier gave the best performance, with correct classification better than 90% for most of the fluorescence classes.***

## IMPACT/APPLICATIONS

We continue to discover new sources of fluorescence in the marine environment. The discovery of intense fluorescence in a wider array of organisms has implications for our interpretation of fluorescence imagery. In an effort funded as part of the Coastal Benthic Optical Properties (CoBOP) program we demonstrated the potential to use multi-wavelength fluorescence imagery generated by the Fluorescence Imaging Laser Line Scanner (FILLS) to perform automated classification of coral reef surfaces (Mazel et al., 2003). In that work we interpreted all brightly fluorescing pixels as being associated with corals, even for very small targets of 1 – 5 pixels. We now know that small brightly fluorescing features can arise from polychaetes, mollusks, crustaceans, fish, or other sources. This will not have a large impact on estimates of percent cover, but could be very important in regard to estimation of numbers of juvenile (i.e., small) corals.

The discovery of fluorescence of methane hydrates may prove to be of some significance. These deposits are of great current interest as potential energy sources. While an optical tool that finds only exposed deposits will be of little use for general exploration, the fluorescence may be of use in studies of the biological characteristics of these unique seafloor features.

The algorithm development results provide guidance for selection of spectral bands for automated benthic classification techniques based on fluorescence measurement or multispectral imaging.

## TRANSITIONS

The LED-based light source for stimulating fluorescence has become a standard product of NightSea, a PSI subsidiary. More than 30 units have been sold into the research community, and additional units to recreational divers. NightSea products, which have largely grown out of ONR-supported research, are now in use at more than 60 scientific institutions worldwide (http://www.nightsea.com/resinst.htm).

## RELATED PROJECTS

I have funding through the NOAA SBIR program to develop fluorescence-based tools for locating and studying coral recruits (juveniles) on natural reef surfaces. The equipment for that specialized application draws on the kind of equipment and experience we have been developing for general fluorescence exploration.

## REFERENCES

Mazel, C. H. 2003. Characterization of Fluorescence in the Marine Environment. ONR FY03 Annual Report.

Mazel, C. H., M. P. Strand, M. P. Lesser, M. P. Crosby, B. Coles, and A. J. Nevis. 2003. High resolution determination of coral reef bottom cover from multispectral fluorescence laser line scan imagery. Limnol. Oceanogr., 48:522-534.

# Characterization of Fluorescence in the Marine Environment

Charles H. Mazel

Physical Sciences Inc., 20 New England Business Center, Andover, MA 01810

phone: (978) 738-8227     fax: (978) 689-3232     email: mazel@psicorp.com

Award Number: N0001402C0130
http://www.nightsea.com

## LONG-TERM GOALS

Our goal is to document the fluorescence of marine organisms, primarily on the seafloor, but also in the water column. We wish to determine the nature and distribution, both geographic and taxonomic, of the effect. The information gained has potential application to mapping and assessment of the sea floor, to studies of fundamental processes in marine biology, and to discovery of novel fluorescing proteins.

## OBJECTIVES

The objectives of the FY05 effort were to continue to conduct fieldwork at several locations; refine techniques for photographing and videotaping fluorescence; make laboratory measurements of fluorescence properties; populate the database of observations, images, and measurements; and conduct deep sea fluorescence exploration from a manned submersible.

## APPROACH

This is largely a field-oriented project, supplemented by laboratory measurement and documentation. SCUBA dives were conducted at a variety of locations, primarily using the LED-based lights perfected during this project to locate instances of seafloor fluorescence. Those lights are also used in other locations such as darkened shipboard rooms to inspect specimens collected during day dives or in net tows. Over the course of this project we have been able to participate in or support three manned submersible projects to explore for fluorescence in deep water in the Bahamas and the Gulf of Mexico. We are concentrating on effects not previously observed or documented by concentrating on habitats that have not been explored much or at all, and on taxonomic groups that have not been examined in detail.

When possible we collect specimens for laboratory measurement of fluorescence excitation and emission spectra. We are organizing our findings in a master database that incorporates observational information (identification, location, depth, appearance, etc.), white-light and fluorescence imagery, and spectral measurements.

Our own observations have been supplemented by those of sport divers using the NightSea equipment spun off from this project. This greatly expands the opportunity to collect fluorescence observations at far-flung locations.

The project is a collaboration with Dr. Michael Lesser, University of New Hampshire, who is funded under a separate award. Dr. Lesser is collaborating in the fieldwork and is carrying out analysis of some of the fluorescing pigments.

**WORK COMPLETED**

SCUBA dives were made at a variety of locations. While the PI for this project was unable to participate in the summer 2005 DeepScope project sponsored by the NOAA Office of Ocean Exploration, he supported the research cruise with the filters that had been used in the 2004 mission to outfit the submersible for fluorescence observation, and has received copies of the images and spectra collected during the mission. Observations were added to the data base as they were received.

**RESULTS**

We will summarize our observations, with example images, for the taxonomic range of organisms commonly found in the sea. We do not treat the plant kingdom, as the sources of fluorescence in that community – chlorophyll and phycoerythrin – are well known and documented, and no novel discoveries were made.

The Phylum Porifera comprises the sponges. Fluorescence occurs, but tends to be relatively weak in comparison to other naturally occurring effects. The fluorescence is in some cases associated with internal structural spicules (Figure 1, left), while in other cases it is distributed diffusely through the tissues or over the surface (Figure 1, right).
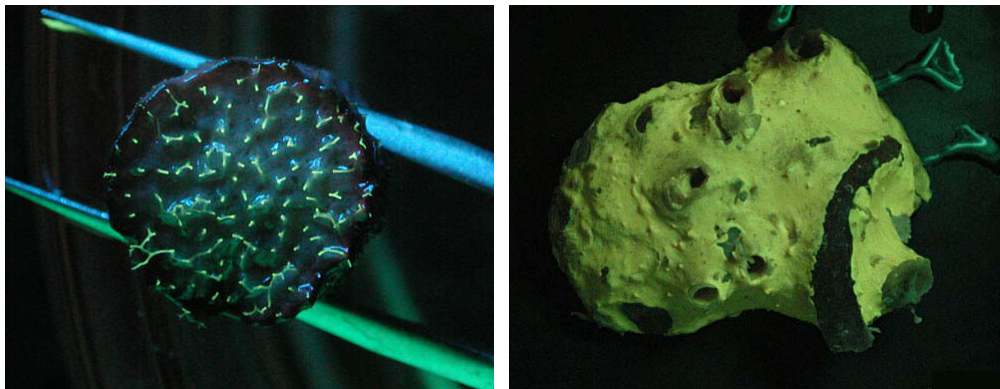


*Figure 1. Photographs of two sponges. In the one on the left only the internal spicules are fluorescent, while in the other the fluorescence is distributed diffusely over the surface.*

The Phylum Cnidaria encompasses a number of commonly occurring marine forms including the siphonophores, hydroids, and fire corals (Class Hydrozoa), the true jellyfish (Class Scyphozoa), and the box jellies (Class Cubozoa). The Class Anthozoa includes the stony corals, anemones, and zoanthids (Subclass Zooantharia (Hexacorallia)) and the sea fans, gorgonians, and soft corals (Subclass Alcyonaria (Octocorallia)).

In the Class Hydrozoa fluorescence in colonial hydroids is common. The Millepora (fire corals) have exhibited only weak fluorescence, if at all. We have had occasion to observe one siphonophore collected in the Gulf of Mexico, and found very small fluorescent spots in several places on the bell.

Figure 2 shows an example of intense green fluorescence in a jellyfish (Scyphozoa). The spectrum of the emission appears identical to that of the green fluorescent protein (GFP) found in corals and originally found in the jellyfish *Aequorea*.
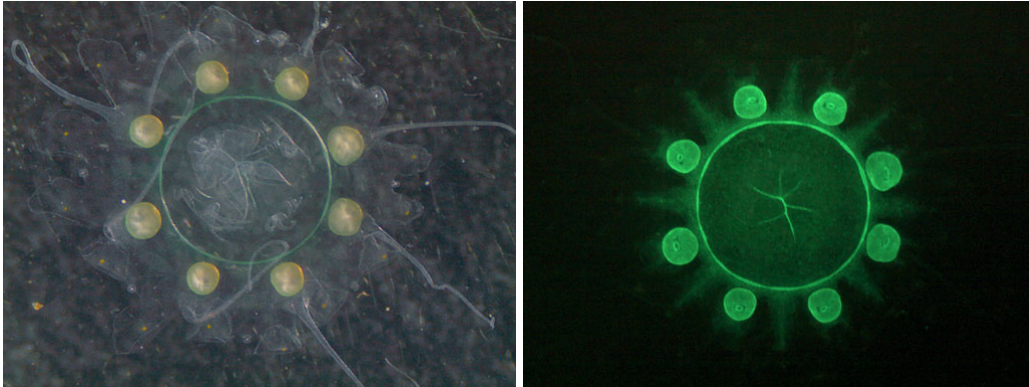


***Figure 2. White light ( left) and fluorescence photographs of a small fluorescing jellyfish collected in surface waters in the Gulf of Mexico. The image shows the intense green fluorescence from the bell ring and from eight radially spaced internal circular structures.***

Fluorescence in the Class Anthozoa is especially widespread and intense. There is such variety in the colors and patterns of coral fluorescence, and photographs have appeared in so many other places, that we only present here a photograph of an anemone collected at a depth of just over 500 m in the Gulf of Mexico (Figure 3). (To see many more examples visit the World Wide Web site http://www.nightsea.com/fluorimages.htm). The source of the fluorescence in the Anthozoa has proven to be proteins belonging to the GFP family. Soft corals (Alcyonacea) in the Caribbean have generally appeared not to have significant fluorescence, while specimens observed off Okinawa were entirely green-fluorescent.



***Figure 3. Fluorescence photograph of an anemone collected from a depth of over 500 m in the Gulf of Mexico. The photograph shows intense green fluorescence in a horizontally striped pattern on the tentacles.***

We have had occasion to look at a few specimens in the Phyla Ctenophora (ctenophores) and Platyhelminthes (flatworms). None of the specimens in those groups observed to date has exhibited fluorescence. We do not have observations in the Phylum Rhyncocoela (Nemertea) (ribbon worms).

In the Phylum Annelida (segmented worms), only the Class Polychaeta are marine. Some have not been fluorescent, while others are intensely fluorescent. The coral-eating fireworm *Hermodice carunculata* in particular has striking yellow, green and orange fluorescence (Figure 4, left). It is interesting to note that in *H. carunculata* the fluorescence is in the body and the bristles (setae) are non-fluorescent, while in other specimens the bristles have been strongly fluorescent and the body non-fluorescent. We have seen many non-fluorescent tubeworms in the Caribbean, but a NightSea customer contributed the observation and photograph of a fluorescing tubeworm in the genus *Dodecacaria* from British Columbia (Figure 5).
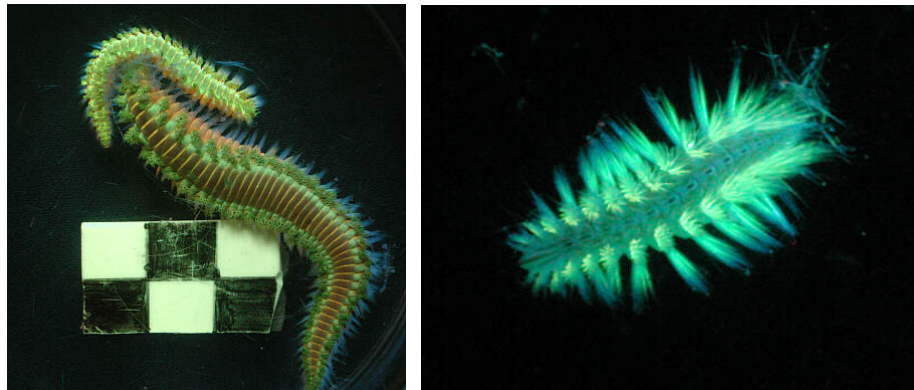


***Figure 4. Fluorescence photographs of the fireworm* Hermodice carunculata *(left) and an unidentified polychaete, both collected in the Bahamas. The fireworm has a pattern of intense orange, green and yellow fluorescence emission in the body, while the other polychaete has a strong green fluorescence from the bristles along the body.***
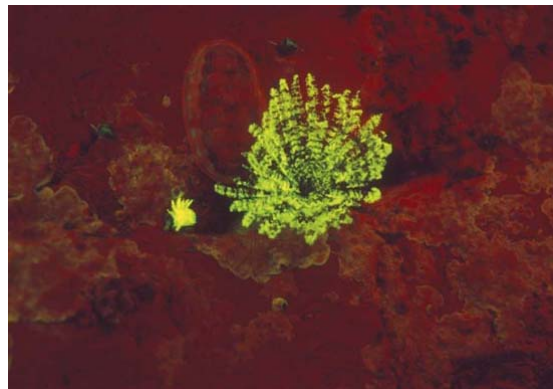


***Figure 5. Fluorescence photograph of a tube worm (polychaete,* Dodecacaria *sp.), British Columbia, Canada. (Photo credit Stuart and Michele Westmorland)***

The Phylum Arthropoda includes the Subphylum Chelicerata, containing the horseshoe crabs (Class Merostomata) and sea spiders (Class Pycnogonida), and the Subphylum Crustacea, comprising a number of classes that include the isopods, amphipods, crabs, shrimp, lobsters, stomatopods, ostracods, copepods, and barnacles. Fluorescence is quite common in this phylum, and it often appears to be associated with the chitinous exoskeleton. Sometimes the fluorescence appears in patterns, while at other times it covers the entire surface. Examples can be seen in the all-over green fluorescence of a picnogonid collected at a depth of 500 m (Figure 6), and in the shrimp, isopod, and crabs collected by SCUBA divers (Figure 7). Figure 8 shows fluorescence photographs of a galatheid crab collected from

deep water in the Gulf of Mexico and of a lobster photographed in the Channel Islands off California. A very interesting pattern has been discovered in some copepods collected from surface waters in the Gulf of Mexico, with weak to no fluorescence in most of the body and intense fluorescence in an elongated appendage (Figure 9). This striking pattern suggests that the fluorescence might serve a visual function in these animals, especially since the females lacked this effect.



*Figure 6. A small (several cm) picnogonid collected at a depth of approximately 600 m in the Gulf of Mexico. A much larger specimen with similar fluorescence was observed and videotaped at depth.*
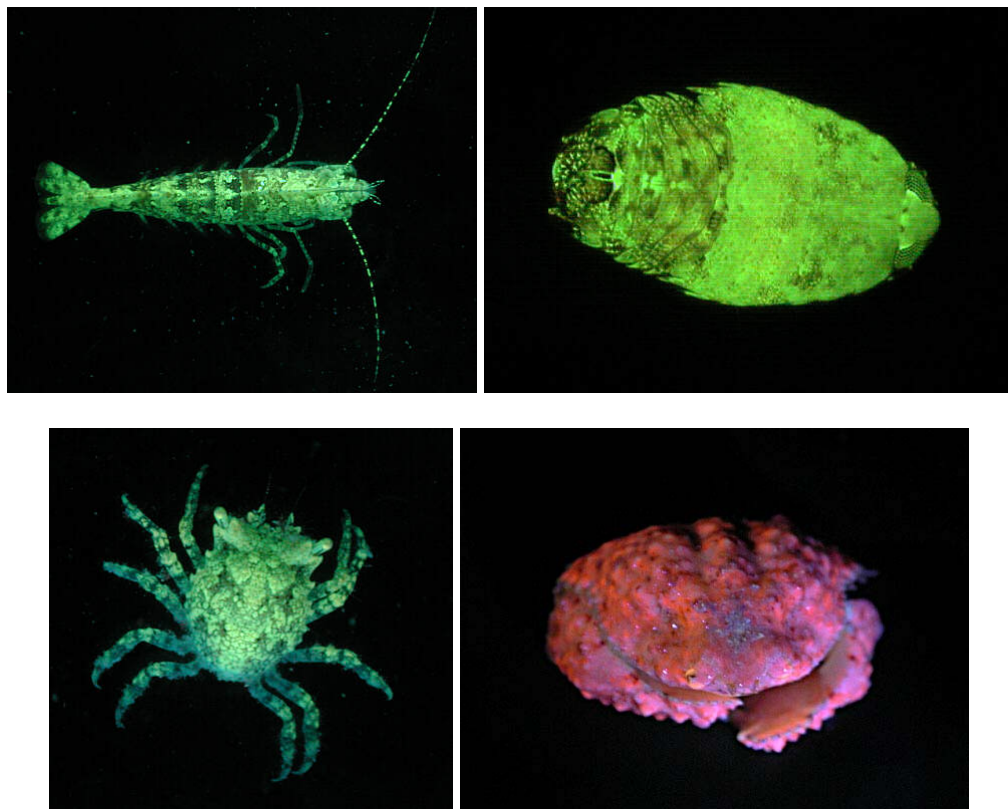


*Figure 7. Fluorescence photographs of a shrimp (top left), an isopod (top right) and of two small (approximately 1 cm) crabs collected in the Bahamas. The shrimp and isopod have intense patterned green fluorescence, as does the crab in the left photograph. The other crab exhibits an all-over red-orange emission.*

*Figure 8. Fluorescence photographs of a deepwater galatheid crab (left) collected at a depth of 500 m in the Gulf of Mexico, and of a California spiny lobster,* **Panulirus interruptus***, photographed by a SCUBA diver at San Clemente, Channel Islands (lobster image courtesy of Chris Grossman).*
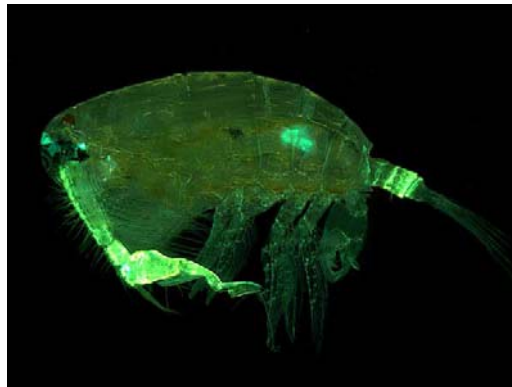


*Figure 9.  Fluorescence photograph of a copepod showing the intense green fluorescence of the elongated appendage (photograph courtesy of Dr. Mikhail Matz).*

The Phylum Mollusca includes the Classes Cephalopoda (octopus and squid), Gastropoda (gastropods, sea slugs, snails, nudibranchs), and Bivalvia (bivalves). We have not yet seen fluorescence in the octopus and squids we have observed. Several different species of nudibranch (shell-less gastropods) have been observed to be fluorescent. Fluorescence is common but by no means universal in mollusks, with some having fluorescence in the shell. The operculum (hard shell closure in the gastropods) is almost always intensely green fluorescent.

The Phylum Echinodermata includes the Classes Crinoidea (crinoids), Stelleroidea (starfish and brittle stars), Echinoidea (sea urchins and sand dollars), and Holothuroidea (sea cucumbers). Fluorescence is common, but not universal, in the crinoids. It is generally absent in the other classes, although we found a weakly red-fluorescent sea urchin deep in the Gulf of Mexico.

The Phylum Chordata includes the important Subphyla Urochordata (tunicates) and Vertebrata. The Vertebrata includes the Classes Osteichthyes, the bony fish, and Chondrichthyes, the sharks and rays (cartilaginous fish). The more time people spend in the water with the lights, the more instances of fluorescence are being discovered in the bony fish, often with very dramatic appearance (Figure 10).

***Figure 10. White-light (left) and fluorescence photographs of a small coral goby collected in the Bahamas. The fluorescence is a vivid orange that runs along the top of the dorsal surface to circles around the eyes.***

We have occasionally had opportunity to point the fluorescence-exciting lights at sharks or sting rays during SCUBA dives without observing any effect. On one occasion during each of the 2004 and 2005 NOAA DeepScope missions a fluorescent shark about a meter long was observed at a depth of about 600 m, and during the latter mission the animal had the courtesy to pose for video images (Figure 11).



***Figure 11. Video frame of a fluorescing shark, the chain dogfish (also called chain catshark, Scyliorhinus retifer) at a depth of about 600 meters in the Gulf of Mexico. The fluorescence pattern corresponds to the coloration pattern that is seen under white light.***

**IMPACT/APPLICATIONS**

The discovery of fluorescence in new groups of marine organisms continues to open doors to new areas of research. The occurrence of fluorescence in the stomatopods (mantis shrimp), for example, was unknown to the primary vision researchers interested in those animals until our discovery, and now it is a regular portion of their research program. A number of the new instances of fluorescence have been found to arise from forms of the green fluorescent protein that is a scientifically and commercially valuable marker, and so may lead to new genetic labeling techniques and products.

As the ONR-inspired NightSea fluorescence equipment becomes more widespread it is beginning to have an impact in a range of scientific studies for discovery, for documentation, and as a core research

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tool. Several publications have cited the use of NightSea lights and/or filters for observing and photographing fluorescence, both in marine and terrestrial organisms. These include:

Gandía-Herrero, F., F. García-Carmona, and J. Escribano. 2005. Floral fluorescence effect. Nature 437:334.

Kahng, S. E., and A. Salih. 2005. Localization of fluorescent pigments in a nonbioluminescent, azooxanthellate octocoral suggests a photoprotective function. Coral Reefs Online First.

Kelmanson, I. V., and M. V. Matz, 2003. Molecular basis and evolutionary origins of color diversity in great star coral *Montastraea cavernosa* (Scleractinia: Faviida). Mol. Biol. Evol. 20:1125-1133.

Piniak, G. A., N. D. Fogarty, C. M. Addison, and W. J. Kenworthy. 2005. Fluorescence census techniques for coral recruits. Coral Reefs Online First.

## TRANSITIONS

The techniques we have developed for exciting and observing fluorescence under ONR sponsorship have become standard products of NightSea LLC (http://www.nightsea.com), a subsidiary of Physical Sciences Inc. The equipment is being used by the research community and by recreational divers. NightSea products are now in use at 80 research and educational institutions in 21 countries (http://www.nightsea.com/resinst.htm). Among these are a number of US government agencies, including two divisions of the Environmental Protection Agency and five different branches of the National Oceanic and Atmospheric Administration (NOAA).

## RELATED PROJECTS

I have Phase 2 SBIR funding from NOAA to develop fluorescence-based equipment, for use both underwater and the laboratory, for locating and studying very small (~1 mm) juvenile corals. The solutions for that specialized application draw on the equipment and expertise we have developed for general fluorescence exploration. Several of the tools we have created in the NOAA program in the past year will have more widespread use just than the specific application to coral recruitment.

## PUBLICATIONS

Mazel, C. Underwater fluorescence photography in the presence of ambient light. Limnology & Oceanography: Methods. [in press, refereed]